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MRI Project No. 2688-B

Contract No. DA-18-106-AMC-120(A)

BIMONTHLY PROGRESS REPORT NO. 6

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Covering the Period

1 February - 31 March 1964

MAY 18 1964

RESEARCH, DEVELOPMENT AND FABRICATION OF AN EXPENDABLE  
BIOELECTROCHEMICAL DETECTOR SYSTEM



MIDWEST RESEARCH INSTITUTE

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Prepared by

Elven K. Bauman

7 May 1964

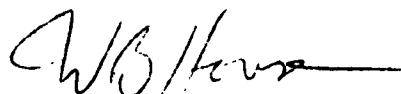
### PREFACE

This report describes the work performed during the bimonthly period ending 31 March 1964 on Contract No. DA-18-108-AMC-120(A).

The experiments were conducted by Mr. Elven K. Bauman, Project Leader, and Mrs. Rosalie Hudson. Dr. L. H. Goodson, Principal Biochemist, assisted in the planning of the experiments and in the evaluation of the results obtained.

Approved for:

MIDWEST RESEARCH INSTITUTE

A handwritten signature in dark ink, appearing to read 'W.B. House', with a long horizontal stroke extending to the right.

W. B. House, Director  
Biological Sciences Division

7 May 1964

#### SUMMARY

This report describes the fabrication of two experimental detectors and preliminary operation of one of these at 4°, 25°, and 60°C. Enzyme hydrolysis at the extreme temperatures was normal, except however, a shift of safe voltage was observed. The voltage resulting from contact of the enzymatically hydrolyzed substrate with the electrodes fell during exposure to high temperature and rose during the low-temperature experiment. Additional studies are now needed to determine the effect of temperature extremes on detector operation. A shift of emphasis from obtaining maximum voltage change to obtaining minimum response time is suggested and suitable experiments are outlined. The installation of an agent aerosol generator at Midwest Research Institute is expected to facilitate these studies.

A glossary of terms is appended to this report.

## I. INTRODUCTION

The objectives of this research program are to design, fabricate and evaluate a sensitive bioelectrochemical detector for various CW agents. The principle upon which this device is to function is the constant current polarographic technique described by Kramer, Cannon, and Guilbault.<sup>1/</sup>

Our efforts during the sixth bimonthly period have been devoted to the fabrication of two experimental detectors and the operation of one of these detectors at 4°, 25°, and 60°C.

More meaningful and less ambiguous terms have been devised to describe the operation of the experimental detector and these expressions are defined in a glossary which is appended to this report. The terms previously used are included in this list to clarify earlier descriptions and discussions.

## II. DETECTOR FABRICATION

Two detector units similar to the line drawing shown as Fig. 3, page 7 in Report No. 5 of this project have been fabricated. One detector employs perforated disc electrodes located above and below the enzyme pad while the second detector incorporates an air-liquid separator and an electrode chamber with two platinum wire electrodes. The enzyme pad holder of the first detector is connected directly to the waste receiver and the separator-electrode chamber is eliminated. Figure 1 of this report is a photograph of two completed units. At the present time an external, line-operated millivoltmeter and recorder are used to monitor the operation of the detector.

Since the air-liquid separator has a liquid holdup volume of about 1 ml. and since the substrate flow rate has been held near 1 ml/min, the minimum response time for this configuration is about 1 min. excluding the time required for the enzyme inhibition to take place. In contrast, the liquid holdup in the disc electrode assembly, is negligible. This is borne out in simulated detector operation during the recovery cycle during which rapid changes of voltage immediately follow the start of the flow of hydrolysis products. Figure 2 is a reproduction of a section of a strip chart made during simultaneous monitoring of both the disc and the wire electrodes in the same

<sup>1/</sup> Kramer, D. H., P. L. Cannon, Jr., and G. C. Guilbault, Anal. Chem., **34**, 842-845 (1962).

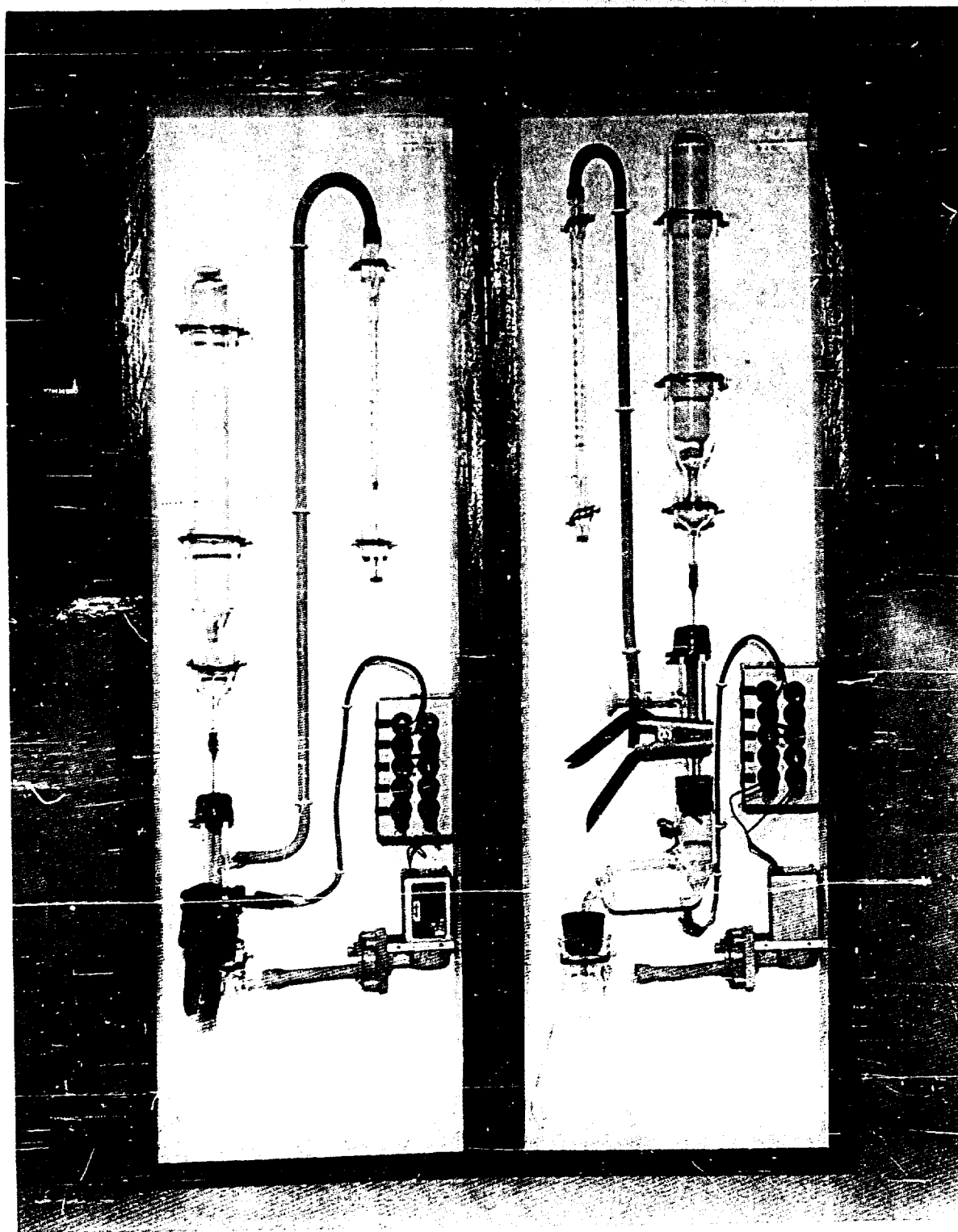


Fig. 1 - Experimental Detectors - Disc Electrode Type (Left)  
and Wire Electrode Type (Right)



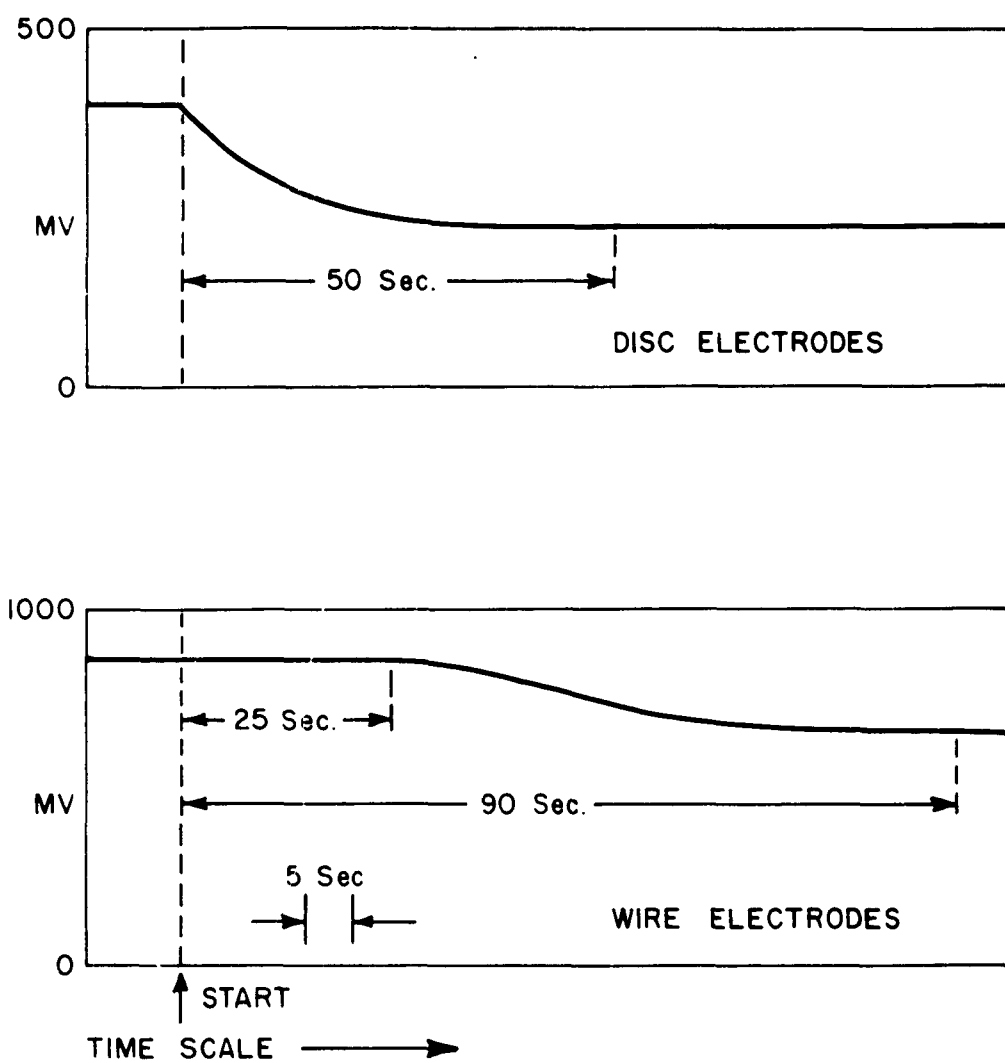


Fig. 2 - Voltage-Time Profiles Following a Change of Liquid Stream from Substrate to Hydrolysis Products. Both Disc and Wire Electrodes Were Monitored Simultaneously in the Same Apparatus.

apparatus. The change of voltage drop between the disc electrodes starts immediately upon the appearance of thiol in the liquid stream and the total change is complete in 50 sec. The initial change of voltage across the wire electrodes appears 25 sec. after the appearance of thiol in the liquid stream. The time required for the change to occur is 65 sec. and the new voltage is not established until 90 sec. after the shift to thiol. In view of the more rapid response obtained with the disc electrodes, they have been used in the remaining experiments described in this report.

### III. DETECTOR OPERATION

#### A. Experimental Operation

At room temperature (ca. 25°C), the disc electrode detector gave results comparable with the results obtained earlier with the experimental apparatus. Table I presents the observations made during several tests.

TABLE I

#### DETECTOR OPERATION AT ROOM TEMPERATURE

<u>Voltage Across Electrode</u>	<u>Run 1</u>	<u>Run 2</u>	<u>Run 3</u>	<u>Run 4</u>
Substrate	380 mv.	475-490 mv.	410-460 mv.	480-525 mv.
Hydrolyzate	170 mv.	130-235 mv.	125 mv.	240-330 mv.
Safe	180 mv.	200-240 mv.	120 mv.	180-210 mv.
Inhibited (eserine)	425 mv.	340 mv.	-	-
Length of Operation	1/2 Hr.	2 Hr.	-	2-1/4 Hr.

While the observed voltages range widely from test to test the difference between substrate voltage and hydrolyzate voltage is consistently near 240 mv. The wide variation of observed voltages between tests is a result of

optimizing the cell current, substrate concentration and substrate flow rate to obtain the maximum change of voltage when the liquid stream is changed from hydrolyzate to substrate. Consistent with this sensitivity any small variation of operating conditions will cause a significant voltage shift.

In all of the tests we used the following conditions:

Substrate:	Butyrylthiocholine iodide
Substrate conc.:	$5 \times 10^{-4}$ M in tribuffer pH 7.4
Substrate flow rate:	1 ml/min
Enzyme:	Serum cholinestrase*
Enzyme conc.:	ca. three units/filter pad
Air flow:	1.8 liters/min
Cell current:	2 $\mu$ a

Fresh substrate was prepared for each test operation.

During operation at 60°C, we observed a lower safe voltage than was observed during room temperature operation. When the detector was returned to room temperature the safe voltage rose to the value normally observed during room temperature operation. In Table II we have tabulated the cell voltage resulting from this test.

Qualitative examination of the substrate for thiol after exposure to 60°C for 1-3/4 hr. did not reveal that a significant amount of autohydrolysis had taken place.

During operation at 4°C, we observed a rise of safe voltage due to the lowering of the temperature. Otherwise the detector continued to operate in a normal manner. The results from this test are tabulated in Table III.

The hydrolysis of the substrate by the enzyme pad in the experimental detector at 4°C was observed to take place. Additional studies will be required to determine other operating characteristics of the detector under these conditions.

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\* Horse serum cholinestrase prepared by Armour and Company and obtained from CRDL.

TABLE II

VOLTAGE DROP BETWEEN DISC ELECTRODES DURING DETECTOR  
OPERATION AT 25° AND 60°C

<u>Temp. (°C)</u>	<u>Condition of Detector</u>	<u>Voltage Drop (mv.)</u>
25	Substrate	400-410
25	Hydrolyzate	150-180
25	Safe	200
60	Safe	90
25	Safe	150

Note: The detector was operated 1/4 hr. at 25°C, 1-3/4 hr. at 60°C, and finally 1/4 hr. at 25°C without any interruption.

TABLE III

VOLTAGE DROP BETWEEN DISC ELECTRODES DURING DETECTOR  
OPERATION AT 25° AND 4°C

<u>Temp. (°C)</u>	<u>Condition of Detector</u>	<u>Voltage Drop (mv.)</u>
25	Substrate	440-460
25	Hydrolyzate	180
25	Safe	130-140
4	Safe	225-240

Note: The detector was operated at 25°C for 1-1/2 hr. and then at 4°C for 1-1/4 hr. without interruption.

These two environmental tests were carried out by placing the apparatus in operation at room temperature and subsequently moving the device into the test chamber. This assured us that the device was operating normally when the test was started. On the other hand the reference voltages refer to the device at room temperature. We plan to rerun these tests at 4°C and 60°C to determine the reference voltages at the test temperature.

#### B. Discussion

The results presented above describing detector operation in excess of 2 hr. and operation at extreme temperatures support the feasibility of producing a prototype field device. These results are based on a limited amount of operation; thus, several aspects of the detector must be investigated with laboratory apparatus before starting to reduce the size and weight of the device.

Early in this program we adjusted the operating conditions to a maximum change of voltage with little regard to the time required for the change of voltage to take place. Under these circumstances any fluctuation of operating conditions such as substrate concentration or operating temperature produces a significant shift in the voltage across the electrodes. With the present apparatus and operating conditions it appears that the response time after inhibition is limited by the rate at which the remaining enzymatically produced thiol can be oxidized to the disulfide. In the past we have provided a maximum of two microamperes to carry out the thiol oxidation. Preliminary experiments in which the available current was doubled gave a much more rapid voltage response following inhibition of the enzyme. A systematic investigation of the effect of cell current will be undertaken.

#### III. FUTURE WORK

We believe the next effort in this program should be an investigation of the relationship between substrate concentration, substrate flow rate and cell current as it affects response time.

Several experiments are planned wherein various substrate concentrations and flow rates will be polarographically observed at several different current levels to select a combination of concentration, flow rate and current that will give an adequate voltage change at a minimum response time and require a minimum of substrate.

The installation and operation of an agent aerosol generator at MRI appears to be prerequisite for studies of the sensitivity of the detector which is being developed. We plan to install a generator capable of producing an air stream containing one gamma of GB per liter at a rate of 2-3 liters per minute. The resulting one gamma per liter stream will permit a quantitative study of the relationship of enzyme concentration to the response time and sensitivity of the detector. A later study will be concerned with response time of the detector to lower concentrations of agents.

During extended operation of the experimental detectors, we have observed a slow upward drift of cell voltage (ca. 10-20 mv/hr). In addition we have found that from 30-60 min. are required for the cell voltage to stabilize when the detector is first placed in operation. To minimize these problems, the use of nonplatinum electrodes should be investigated. The possible use of dissimilar electrodes should also be considered.

Upon completion of these several investigations, the emphasis of the program will be directed toward the reduction of the size and weight of the device and incorporation of suitable solid state circuitry for monitoring the voltage drop across the electrodes.

## APPENDIX

### GLOSSARY OF TERMS

Enzyme Pad - a urethane foam pad containing freeze-dried starch gel and enzyme.

Hydrolyzate Voltage - (previously, safe reference voltage) - the voltage drop across the electrodes when the electrodes are in contact with prehydrolyzed substrate and there is no enzyme present. These conditions simulate a safe situation in which the enzyme completely hydrolyzes the substrate stream.

Inhibited Voltage - (previously, inhibited voltage) - the voltage drop across the electrodes when the electrodes are in contact with unhydrolyzed substrate as a result of inhibition of the immobilized enzyme. This voltage indicates the accumulation of sufficient enzyme inhibitor from the air stream to inactivate the enzyme. In theory this voltage will be equal to or slightly less than the substrate voltage when complete inhibition has occurred.

Recovery Time - the time required for the cell voltage to reach a new and stable value following simulated or actual reactivation of the immobilized enzyme.

Response Time - the time required for the cell voltage to reach a new and stable value following simulated or actual inhibition of the immobilized enzyme.

Safe Voltage - (previously, operating voltage) - the voltage drop across the electrodes when the electrodes are in contact with hydrolyzed substrate (hydrolyzate) produced by the action of the immobilized enzyme. This voltage indicates that the substrate stream is being hydrolyzed by the immobilized enzyme and that the air stream is free of inhibitors. In theory this voltage will be equal to or slightly greater than the hydrolyzate voltage.

Reference Voltages - the substrate hydrolyzate voltages which simulate sampling air containing an inhibitor and clean air, respectively.

Simulated Operation - alternating the liquid stream in the detector between unhydrolyzed and prehydrolyzed substrate to simulate the instantaneous inhibition and reactivation of the enzyme. An enzyme pad is not used during simulated operation.

Starch Pad - a urethane foam pad containing freeze-dried starch gel but not containing enzyme.

Substrate Voltage - (previously, alarm reference voltage) - the voltage drop across the electrodes when the electrodes are in contact with unhydrolyzed substrate and there is no enzyme present. These conditions coincide with the alarm situation in which the enzyme is completely inhibited.

Voltage or Cell Voltage - the voltage drop measured across the electrodes.



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